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NUTRITIONAL HEALTH OF ADOLESCENT FEMALES: ZINC, COPPER AND LIPID STATUS

*The University of North Carolina at Greensboro* PH.D. 1985

# NUTRITIONAL HEALTH OF ADOLESCENT FEMALES: ZINC, COPPER AND LIPID STATUS

by

James Donald Kinard

A Dissertation submitted to
the Faculty of the Graduate School at
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Approved by

Dissertation Adviser

APPROVAL PAGE

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KINARD, JAMES DONALD, Ph.D. Nutritional Health of Adolescent Females: Zinc, Copper, and Lipid Status. (1985) Directed by Terry L. Bazzarre. Pp. 69.

This dissertation research investigated the nutritional health of adolescent females in both a cross-sectional study and a longitudinal study. Cross-sectional investigations included dietary, plasma, and erythrocyte zinc and copper. Cross-sectional and longitudinal investigations included total cholesterol, HDL-cholesterol, body weight, a height weight index, dietary fat, and dietary cholesterol. Dietary intake was assessed by two 24-hour recalls and a food frequency questionnaire. Participants in the study were 92 adolescent females, 14-16 years of age, of which 38% were black.

Dietary zinc intake for the adolescent females in this study was 81% of the Recommended Dietary Allowance for this population. A significant difference ($p = 0.001$) in zinc intake per 1000 kcals was observed between the races. Ten percent of the adolescent females were marginally deficient in zinc on the basis of plasma zinc levels less than 65 ug/100 ml. Twenty-two percent of the adolescent females were marginally deficient in zinc on the basis of erythrocyte zinc concentrations of less than 8.0 ug/gm of RBC.

Dietary intake of copper for the adolescent females ($1.4 \pm 0.7$ mg/day) was less than the

suggested intake for healthy adults. None of the adolescent females were marginally deficient in copper on the basis of plasma copper concentrations.

Erythrocyte copper concentrations were inversely correlated with plasma zinc ($r$ = -0.32, $p$ = 0.002) and the ratio of zinc to copper in plasma ($r$ = -0.30, $p$ = 0.005). Erythrocyte copper was inversely correlated with the ratio of dietary zinc to copper ($r$ = - 0.21, $p$ = 0.04).

Total cholesterol was significantly higher in the 16-year-old females than the 14-year-old females ($p$ = 0.04) and HDL-cholesterol was also significantly higher in the 16-year-old females ($p$ = 0.02). Total cholesterol was not significantly different between the races, however, HDL-cholesterol was significantly ($p$ = 0.0009) higher in the black population.

A significant positive relationship ($r$ = 0.26, $p$ = 0.012) was observed between the total score on the food frequency questionnaire and the total dietary fat intake estimated from the average of two 24-hour recalls. A similar relationship was observed between total dietary animal fat and total score on the food frequency questionnaire ($r$ = 0.32, $p$ = 0.002).

Total cholesterol and HDL-cholesterol increased significantly for the adolescent females during the two-year period. Total cholesterol increased 19 mg/100 ml ($p$ < 0.0001) and HDL-cholesterol

increased 6 mg/100 ml ($p < 0.0001$). Changes in body weight were negatively correlated with changes in HDL-cholesterol during the two-year period ($r = -0.23$, $p = 0.05$). A significant positive relationship ($r = 0.34$, $p = 0.04$) was observed between changes in the Quetlet Index and changes in total cholesterol for the females who were 14 years old in 1981.

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# CHAPTER 1

# INTRODUCTION

Atherosclerosis and subsequent complications of this disease are the leading cause of morbidity and mortality in the United States (Christensen et al., 1980). Research findings suggest that atherogenesis begins in childhood (Stein, Clueck, & Morrison, 1981). Abnormal lipid levels are closely linked to the development of coronary heart disease (CHD); however, the role or diet, especially that of trace minerals in the development of lipid abnormalities and subsequently CHD is unclear. A longitudinal and cross-sectional study of dietary patterns in relation to lipid profiles among adolescent females may provide a better understanding of the role that nutrition plays in the premature development of CHD.

# CHAPTER 2

# REVIEW OF LITERATURE

This review of literature will address three major areas regarding the nutritional health of adolescents. The first section will focus on zinc and copper nutriture during adolescence. The second section will focus on the relationship between zinc and copper levels and cardiovascular disease. The third section will focus on the prevalence of risk factors for cardiovascular disease during adolescence.

## Zinc and Copper Nutriture in Adolescence

Adolescence is a period of increased physical growth which ranks second only to the growth rate during infancy (Lee, 1978). Increased physical growth results in an increased demand for calories and nutrients to support optimal growth. Adolescent females are often recognized as a high risk group not only because of an increased growth rate but also because of social pressures and changes in food habits that may occur during this period.

Relatively little scientific information is known about the nutritional health of adolescent females, especially with regard to trace mineral status. Recommended Dietary Allowances (1980) for the adolescent are primarily based on extrapolation of the allowances for adults and young children. Greger et al. (1978a) surveyed 367 adolescent females in the U.S. and found that these girls consumed only 60 to 75% of the Recommended Dietary Allowances for iron and zinc. Of these 367 girls, blood samples were obtained on a subsample of 102 girls. Greger observed that 3% of the adolescent females had serum zinc levels below 70 ug/100 ml and 4% of the females had serum copper levels below 70 ug/100 ml. Greger et al. (1978) concluded that many of the girls consumed less than optimal levels of dietary zinc and copper and that 3 to 4% of the girls could be classified as marginally deficient with regard to zinc and copper status. Sandstead (1982) has suggested that mild dietary zinc deficiency is not uncommon in both under-developed and industrialized nations and that this deficiency may be related to the low availability of zinc in foods.

Requirements for zinc increase for children entering adolescence as a result of the accelerated growth rate that occurs during this time period

(Sandstead, 1982). Zinc deficiency has been reported among adolescents in Egypt, Iran and the U.S. (Hambidge, 1977). Zinc requirements for the adolescent female, based on balance studies, are 12.3 mg/day before menarche and 11.9 mg/day after menarche (Sandstead, 1982).

Copper and zinc are mutually antagonistic during absorption (Fischer, Giroux, Belonje & Shah, 1980). Greger, Zaikis, Abernathy, Bennett, and Huffman (1978b) noted an increase in fecal copper excretion with higher zinc intakes during balance studies using adolescent females. A significant increase ($p < .005$) in fecal copper excretion was observed in adolescent females consuming 14.7 mg of zinc compared to those who consumed 11.5 mg daily. In contrast, Tapper, Hinners, and Ritchey (1980) reported no differences in copper balance among adult women consuming 2.0 mg of copper and either 8, 16, or 24 mg of zinc daily. The Recommended Dietary Allowances do not allow for differences in zinc availability in the food supply or for the effects that other nutrients may have on zinc absorption and requirements (Sandstead, 1982).

Butrimovitz and Purdy (1978) investigated zinc nutrition and growth in a childhood population of 156 volunteers and found that the lowest plasma zinc

concentrations occurred during puberty at age 15 in females. The lowest plasma zinc concentrations occurred approximately one year beyond the point of maximum growth in the female population. Butrimovitz and Purdy (1978) suggested that decreases in plasma zinc concentrations may reflect depletion of zinc stores which follow periods of rapid growth.

Zinc/Copper Levels and Cardiovascular Disease

Klevay (1975) proposed that the zinc/copper ratio in the diet is a major determinant of coronary heart disease in humans after repeatedly producing hypercholesterolemia in rats fed a diet with a high ratio of zinc/copper. Since Klevay (1973) developed his hypothesis, many researchers have attempted to validate this theory in both human and rat experimental studies. No conclusions have been reached since researchers have obtained widely varying results depending on the experimental design.

Fischer, Giroux, Belonje and Shah (1980) evaluated the effects of variable amounts of dietary zinc (7.5, 10, 30, 45, 60 mg/kg) and copper (1.5, 3.0, 6.0 mg/kg) for 15 weeks in 168 adult rats and concluded that marginal, adequate, or excessive levels of dietary zinc and copper had no significant effect on cholesterol

metabolism (Table 1). Similarly, Woo and Gibbs (1981) concluded that 12 weeks of variable amounts (10, 100, and 500 mg/kg) of dietary zinc with constant intakes of copper (15 mg/kg) had no significant effect on serum cholesterol, HDL-cholesterol or serum triglyceride concentrations in rats. Petering, Murthy and O'Flaherty (1977) evaluated the effects of 110 days of variable levels of dietary zinc (2.5, 10, 40 ug/ml of drinking water) and copper (0.0, 0.25, 2.0, 10.0, ug/ml of drinking water) on lipid parameters in 72 male rats and found an inverse relationship ($r$ = -0.47; $p < 0.001$) between serum copper and serum cholesterol. No correlation was observed between serum or dietary zinc and serum cholesterol in the male rats. Caster and Doster (1979) evaluated the effects of 27 days of variable levels of dietary zinc (11, 29, 100ppm) and copper (0.0, 1.3, 5.1 ppm) in 27 young rats and found no correlation between the zinc/copper dietary ratio and plasma cholesterol. Caster and Doster (1979) concluded that some other factor in cereal diets must be responsible for increases in plasma cholesterol of rats fed varying levels of zinc and copper.

In contrast to previous research with adult male rats, Koo and Williams (1981) observed a significant reduction ($p < 0.05$) in total serum cholesterol (23 mg)

Table 1

Effects of Alterations in the Dietary Zinc/Copper Ratio on Lipid Metabolism in Rats

| Researchers | Experimental Treatment | Time Period | Effect |
|---|---|---|---|
| Fischer et al., (1979) | low, adequate or excessive Zn & Cu | 15 wk | none |
| Woo & Gibbs, (1981) | marginal or high Zn with adequate Cu | 12 wk | none |
| Petering et al., (1977) | low, adequate or excessive Zn & Cu | 16 wk | Cu & TC inversely correlated |
| Caster & Doster, (1979) | low, adequate or excessive Zn & Cu | 4 wk | none |
| Fischer et al., (1980) | low, adequate or excessive Zn & Cu | 15 wk | none |
| Koo & Williams (1981) | Zn deficiency | 12 wk | decrease in HDL-C & TC |

Note. See text for specific mineral levels.

and HDL-cholesterol (17 mg) in 12 rats fed zinc deficient diets (0.37 ppm zinc). Koo and Williams (1981) also noted a significant correlation ($r$ = 0.81, $p$ < 0.05) between total serum cholesterol and the ratio of zinc/copper in serum. Further, Koo and Williams (1981) noted that the reduction in total cholesterol with zinc depletion was primarily due to a reduction in HDL-cholesterol suggesting an important role between zinc nutriture and cardiovascular health.

## Trace Minerals and Mechanisms for Cardiovascular Disease

The role of zinc and copper in cardiovascular health is unclear, but may be related to the possible effects of these minerals on lipoprotein fractions. Allen and Klevay (1980) noted a reduction in both high and low density lipoproteins with copper deficiency in 7 adult rats, but the percent plasma cholesterol associated with LDL was significantly ($p$ < 0.001) increased. Koo and Turk (1977) observed that a zinc deficiency in rats resulted in defective intestinal transport of triglyceride primarily due to a decrease in mucosal protein synthesis. Lau and Klevay (1981) noted a 22-32% reduction in plasma lecithin cholesterol acyl transferase (LCAT) activity in copper deficient rats.

Low LCAT activity in humans has been associated with increased risk of ischemic heart disease (Lau and Klevay, 1981).

## Human Studies

A few studies have focused on the role of zinc and copper in cardiovascular health using humans as experimental models (Table 2). Freeland-Graves, Friedman, Hans, Shorey and Young (1982) assessed the effects of zinc supplementation on plasma HDL-cholesterol in normal adult women and noted a decrease in HDL-cholesterol only with a zinc supplementation of 100 mg/day (about 7 times the adult RDA). Similarly, Hooper, Visconti, Garry and Johnson (1980) reported that zinc supplementation of 160 mg/day for 6 weeks lowered HDL-cholesterol in 12 men. Freeland-Graves, Friedman, Han and Shorey (1980) observed plasma cholesterol levels to be inversely related to dietary copper intakes ($r$ = -0.21, $p < 0.009$) when altering dietary zinc/copper ratios in the diets of 32 women. In conclusion, the role of zinc and copper in relation to lipid metabolism and cardiovascular health is a controversial one. Further research with humans and experimental animals is needed to evaluate the role of zinc and copper in cardiovascular health.

Table 2

Effects of Alterations in the Dietary Zinc/Copper Ratio on Lipid Metabolism in Humans

| Researchers | Experimental Treatment | Sample | Effect |
|---|---|---|---|
| Freeland-Graves et al., (1982) | zinc supplement 100 mg/day | 24 adult women | decrease in HDL-C |
| Hooper et al., (1980) | zinc supplement 160 mg/day | 12 adult men | decrease in HDL-C |
| Freeland-Graves, (1980) | Alteration of dietary Zn/Cu | 32 adult women | TC & Cu related |

## Prevalence of Risk Factors in Adolescence

A high fat diet apparently affects serum lipids and presumably cardiovascular health as early as 12 to 14 years of age. Roberts (1978) reported that 4.6% of the U.S. population between 12 and 17 years of age have significant signs of increased cardiovascular risk with a higher prevalence among low income blacks of the South. Kies, Lin, Fox and Korslund (1975) noted differences in serum fatty acid patterns of adolescent boys consuming varying levels of dietary fat. Lee (1978) noted a high incidence of elevated serum cholesterol levels in a study of 118 Kentucky teenagers.

## Lipid Changes During Adolescence

During adolescence there is a very rapid rise in the amount of fatty streaking within the arteries (Heald, 1979). Fatty streaking is one of the beginning stages of atherosclerosis which is the major cause of ischemic heart disease throughout the world (Heald, 1979). While the period of adolescence is associated with increased fatty streaking, some researchers have shown that lipid concentrations within the blood may

actually decrease during this period. Based on a cross sectional investigation of cardiovascular disease risk factors of 625 adolescents, Orchard, Rodgers, Hedley and Mitchell (1980) reported that HDL cholesterol decreased significantly ($p < 0.001$) during adolescence with increasing height and sexual maturity in boys but not in the girls. These data may account for sex differences in CHD incidence which are manifested later in life.

Plasma cholesterol levels decreased in both adolescent males and females evaluated in the Lipid Research Clinics Program Prevalence Study. Plasma cholesterol for the 2,080 white females 10-14 years of age was 159.6 $\pm$ 24.1 mg/100 ml and for the 1,911 white females 15-19 years of age, the plasma cholesterol level was 156.6 $\pm$ 26.4 mg/100 ml (Rifkind et al., 1979). Total cholesterol decreased during adolescence in white females from 161 $\pm$ 24 mg/100 ml ($n$ = 536) at age 12 to 157 $\pm$ 25 mg/100 ml ($n$ = 441) at age 14 and to 155 $\pm$ 27 mg/ 100 ml ($n$ = 675) at age 16 (Christensen et al., 1980). Total cholesterol in the black females remained essentially unchanged during the ages of 12-16 years with values 161 $\pm$ 26 mg/100 ml ($n$ = 128) at age 12; 161 $\pm$ 29 mg/100 ml ($n$ = 80) at age 14; and 162 $\pm$ 27 mg/ 100 ml ($n$ = 192) at age 16. Total cholesterol levels

for the black population were consistently higher than the white populations from age 6 to 19 ($p < 0.001$). Similarly, higher mean levels of serum cholesterol were reported in the Bogalusa Heart Study ($n = 3,446$) among black children than white children, 170 mg/dl vs. 162 mg/dl, $p < 0.0001$ (Frerichs, Srinivasan, Webber, & Berenson, 1976). Serum cholesterol also decreased in children around 11-12 years of age but this reduction was more pronounced among boys than girls. The mean total cholesterol concentration was 163.7 $\pm$ 24 mg/100 ml among black girls, 12 years of age ($n$=77), and 162.9 $\pm$ 28 mg/100 ml among white girls, 12 years of age ($n$ = 129). The mean total cholesterol concentration was 166.6 $\pm$ 28.5 mg/100 ml among black girls, 14 years of age ($n$ = 63) and 157.7 $\pm$ 26 mg/100 ml among white girls, 14 years of age ($n$ = 69).

Significantly higher ($p < 0.0001$) levels of alpha lipoprotein were observed among black children (436 mg/100 ml) than white children (384 mg/100 ml) of those children participating in the Bogalusa Heart Study (Srinivasan et al., 1976). White children in the Bogalusa Heart Study had significantly higher pre-B-lipoprotein levels than black children (40 mg/100 ml vs. 33 mg/100 ml, $p < 0.002$).

Lee (1967) observed longitudinal changes in total

serum cholesterol of 16 girls over a 10 year period and found that cholesterol decreased in this population during adolescence. Total cholesterol levels decreased a mean of 9% among 12 of the 16 girls studied, whereas total cholesterol remained the same in 4 of the 16 girls studied.

In conclusion, very few studies have investigated longitudinal changes in total cholesterol and HDL-cholesterol during adolescence. Cross sectional studies suggest that total cholesterol decreases during adolescence, and that racial differences exist for levels of total cholesterol and the cholesterol subfractions.

## Methods of Assessing Food Intake

Several methods exist for assessing food intake of populations. The most accurate method for collecting dietary information is the seven day food record, however, this method is also the most expensive and time consuming (Bazzarre & Myers, 1978). Food frequency questionnaires and diet histories provide the best assessment of usual intake but are limited in providing quantitative information about specific nutrients (Bazzarre & Myers, 1978). An advantage of food frequency questionnaires aside from identifying usual

intake and being relatively inexpensive is that their use eliminates the variance associated with day to day changes in eating (Axelson & Csernus, 1983). Twenty-four hour dietary recalls provide quantitative information about specific nutrients but are not as accurate as food records.

Greger and Etnyre (1978) evaluated the validity of 24-hour recalls of adolescent females by comparing the results from the recall to previously weighed and measured food intake. They concluded that the dietary recall for groups of individuals provided valid estimates of energy, protein, calcium, and zinc intake but provided invalid estimates for vitamin A, ascorbic acid, thiamin, riboflavin, niacin, and iron intake. Rasanen (1979) compared the 24-hour recall to the dietary history interview in 1,033 children. The author concluded that neither method was adequate for assessing an individual child's intake; however, she concluded that two 24-hour recalls repeated at different times would decrease the respondent burden and increase the reliability of the information for surveys of groups of individuals.

# CHAPTER 3

# EXPERIMENTAL DESIGN

## Statement of Purpose

This dissertation research was part of a research project designed to evaluate the nutritional health of adolescent females in the Southeastern United States. Two components were included in this dissertation research, a cross-sectional component and a longitudinal component. The first component was designed to evaluate the relationships of zinc and copper to lipid measures in a cross-sectional manner. The second component of this dissertation research was designed to evaluate the relationship of dietary patterns to lipid changes over a two-year period.

## Objectives

1. To measure nutrient intake using two 24-hour recalls and a food frequency questionnaire.
2. To measure blood levels of zinc and copper.
3. To measure total and HDL-cholesterol plasma concentrations.

4. To determine associations between zinc copper nutriture, and HDL-cholesterol and total cholesterol.

5. To compare the use of two 24-hour recalls with a food frequency questionnaire in determining associations between diet and blood lipids.

## Hypotheses

1. No statistically significant differences will be observed in zinc and copper status according to (a) race or (b) age.

2. Less than 5% of the adolescent females will exhibit marginal zinc status as evidenced by low plasma levels (<65 ug zinc/100 ml of plasma), low red blood cell levels (<8.0 ug zinc/g of RBC) or low dietary levels (<2/3 of zinc RDA).

3. Less than 5% of the adolescent females will exhibit marginal copper status as evidenced by low plasma levels (<70 ug copper/100 ml of plasma), or low dietary levels (<2/3 of suggested copper intake).

4. Zinc and copper dietary intakes will not be correlated with plasma and red blood cell zinc and copper concentrations.

5. Statistically significant differences between the races will be observed in HDL-cholesterol.

6. Less than 5% of the adolescent females will

exhibit elevated cholesterol concentrations (>95th percentile for the Lipids Research Clinic Guidelines).

7. HDL-cholesterol and total cholesterol concentrations will be correlated with dietary, plasma and red blood cell zinc and copper levels.

8. Changes in total cholesterol levels during the two year study period will be correlated with changes in the Quetelet Index.

## Sample Selection and Data Collection

Ninety-two adolescent females from the Guilford County, North Carolina, region were recruited in 1983 from the original population of 189 females. These individuals previously participated in the Southern Regional Nutrition Project: Nutritional Health of Adolescent Females (S-150) in 1981. The objectives of the regional S-150 study were to assess the nutritional health of adolescent females in the southern region and to relate the nutritional health of adolescent females to socioeconomic factors, food habits, nutritional knowledge, behavioral characteristics, and physiological development. The longitudinal assessment of lipid changes included only those participants who returned

for the two year follow-up. Participants in the program were free to withdraw from the study at any time and informed consent was obtained from each girl (see Appendix A).

Subjects were recruited through the public school systems of Greensboro and Guilford County by means of flyers distributed in the classrooms. Interested students responded by either returning a postcard or by calling the center at UNC-Greensboro.

Subjects recruited the study had no diagnosed medical disorders nor were they suffering from a disease which would prohibit their participation in either 1981 or 1983. Sixty-two percent of the sample was white and 38% was black. Fifty-three percent of the sample in 1983 was 14 years old and 47% of the sample was 16 years old. The girls included in the study could participate regardless of income but attempts to include participants from low incomes were given special emphasis.

All procedures (i.e. recruitment procedures, consent forms, and guidelines for participation of the subjects) were approved by the Human Subjects Review Committee at UNC-Greensboro. The principal investigator for S-150 trained graduate students from the Department of Foods and Nutrition in data collection methods. The

interviewers were trained according to the standards summarized in 1981 Project Procedural Guidelines and Manual for Interviewers.

After completion of training, pairs of trained interviewers visited the subjects' homes. Consent forms were signed by the subject's parent or guardian before the interview took place. During the home visit, questionnaires administered to the parent or quardian included a subject's medical history questionnaire and a sociodemographic questionnaire. Other questionnaires administered to the parent or quardian included internal/external locus of control, nutrition knowledge, an attitude inventory, and a familarism scale.

Data collected from the female participant at the home included a 24-hour recall, a self-esteem scale, an internal/external locus of control scale, a familiarism scale, and a food frequency scale. The home interviews took approximately one and a half hours to complete. A second interview was scheduled for the following Saturday at UNC-Greensboro.

Information collected at UNC-Greensboro included a detailed medical history, a 24-hour recall, a food frequency questionnaire, a nutrition knowledge questionnaire, and a physical activity inventory. Sexual maturity was assessed on Tanner's Sexual Maturity

Scale. Anthropometric measurements and blood pressure measurements were also recorded during the Saturday morning.

## Dietary Methods

Two 24-hour recalls were used to assess dietary intakes of the group with regard to zinc, copper, total fat, saturated fat, polyunsaturated fat, cholesterol and caloric intake. The 24-hour recalls were collected approximately two weeks apart, one recall taking place at the home of the volunteer and another at the Nutrition Research Center, UNC-Greensboro. A food frequency questionnaire was designed to investigate the intake of high cholesterol and/or high saturated fat foods (see Appendix B). The nutrient intake measured by the two 24-hour recalls were calculated with the Nutritional Analysis System developed and maintained by the Department of Experimental Statistics, Louisiana State University.

## Food Frequency Questionnaire

The food frequency questionnaire developed for this dissertation research was designed to investigate the intake of high saturated fat and/or high cholesterol dietary intake. Only those foods known to contribute 5

grams or more of saturated fat or those foods known to contribute 20 milligrams or more of cholesterol per average serving size were included in the food frequency questionnaire. Foods selected for the food frequency questionnaire were chosen from the tables developed by Patten, (1976). Five possible frequencies in consumption of these foods were available for the participants to check: (1) Eaten two or more times daily, (2) Eaten once daily, (3) Eaten three or more times per week, (4) Eaten at least once a week and (5) Eaten seldom or never. Thirty-six different food items were included on the food frequency questionnaire, all food items containing at least five gms of saturated fat or 20 mg of cholesterol per average serving. Total scores on the food frequency questionnaire for each individual were computed by suming the values recorded for each individual food item. Scores were reversed after coding so that a high score would indicate a relatively high consumption of saturated fat or cholesterol compared to a low score. The highest possible total score that could be obtained on the food frequency questionnaire was 180 and the lowest possible total score attainable was 36.

## Biochemical Procedures

Approximately 35 mls of blood were obtained from each of the girls following a 12-hour fast. The Vacutainer System (Becton Dickinson) using mineral-free collection tubes was used for plasma and red blood cell zinc and copper determinations in order to avoid possible trace mineral contamination. Plasma and red blood cell aliquots for zinc and copper determinations were frozen for later analysis.

## Plasma Zinc

Plasma zinc levels were measured by the use of flame atomic absorption spectrophotometry according to the procedure of Butrimovitz (1977). Plasma stored in polypropylene containers was thawed to room temperature and centrifuged at a slow speed (1000 rpm) in order to remove protein colloidal suspensions. The plasma was then diluted 1:5 by combining 0.5 of plasma with 2 ml of distilled-deionized water. Diluted plasma samples were aspirated into the Instrumentation Model 441 Spectrophotometer. A regression curve using 5 standards (50, 100, 150, 200, 250 ug/100 ml) developed from Fisher Scientific Certified Zinc Standards, double distilled deionized water and glycerol, was used in estimating zinc concentrations in plasma. An assayed control

sample was used to ensure the accuracy of the method and pooled plasma samples were analyzed every 15 samples to detect any possible shifts in the zinc curve.

## Plasma Copper

The method of Butrimovitz (1977) for plasma zinc analysis was adapted for plasma copper determination. Plasma stored in polypropylene containers was thawed to room temperature and centrifuged at a slow speed (1000 rpm) to remove protein colloidal suspesions. The plasma was then diluted 1:5 by combining 0.5 ml of plasma with 2 ml of distilled-deionized water. Diluted plasma samples were aspirated into the Instrumentation Model 441 Atomic Absorption Spectrophotometer. A regression curve using 5 standards (50, 100, 150, 200, 250 ug/100 ml) developed from Fisher Scientific Certified Copper Standards, distilled-deionized water and glycerol, was used in estimating copper concentrations in plasma. An assayed control sample was used to ensure the accuracy of the method and pooled plasma samples were analyzed every 15 samples to detect any possible shifts in the copper curve.

## Red Blood Cell Zinc and Copper

One gm of RBC was accurately weighed into a 100 ml acid-washed beaker. Five ml of nitric Acid and 2 ml of sulfuric acid were added to each beaker and covered with a watch glass. Samples were allowed to dissolve for 30 minutes after which time samples were heated to 300°F. Gas bubbles evolved from the solution with the production of brown fumes. Heat was applied for 1 and 1/2 to 2 hours until brown fumes dissipated. Watch glasses were removed from beakers and evaporation of nitric acid took place with the production of white fumes. Samples were removed from heat after samples turned brown. Samples were allowed to cool and 1 ml of 50% peroxide was added to each beaker. Samples were again heated to 300°F and an oxidation reaction occurred resulting in bubbling of the sample. Samples were further heated 1 to 2 hours at 500°F until peroxide and water were driven off. Peroxide was again added to the samples and reheating took place until water and peroxide were driven off. Usually 3-4 ml of peroxide were added to the samples over a 8-10 hour period. The resultant product was a pale yellow or white crystal product.

Samples were cooled and covered with Parafilm until dilution occurred. Samples were later heated and a 3

and 1/2 ml aliquot of deionized, distilled water was added to dissolve the residual material. The diluted solution was removed from beakers with acid washed pipets and placed in a 5 ml volumetric flask. Samples were brought to volume with the addition of deionized distilled water and transferred to 7 ml polypropylene containers. A further 1:2 dilution of samples was made for zinc analysis by transferring 2.5 ml of the solution into another polypropylene contained and diluting this aliquot with an equal amount of deionized distilled water. One blank solution was included with the ashing procedure to detect any contribution to zinc or copper concentrations from sulfuric, nitric acid or peroxide solutions. Samples preanalyzed for zinc and copper were also included with the ashing procedure in order to ensure accuracy of the method.

## Percentage Error

Percentage errors associated with each of the analytical methods were computed for plasma zinc, plasma copper, RBC zinc, and RBC copper. The percentage error associated with pooled plasma samples for the plasma zinc analysis was 5.12%. The percentage error associated with pooled plasma samples for the plasma copper analysis was 6.08% The percentage error

associated with pooled RBC samples for the RBC zinc analysis was 2.46%. The percentage error associated with two pooled RBC samples for RBC copper analysis was 3.92%.

## HDL- and Total Cholesterol

HDL-cholesterol and total cholesterol were analyzed on fresh samples using the Liebermann Burchard manual method (Wybenga & Inkpen, 1974). The accuracy of HDL-cholesterol and total cholesterol determinations was assessed by analyzing assayed control samples obtained from the Fisher Scientific Company. Pooled preanalyzed samples were also analyzed with each run of unknowns to ensure the accuracy of the method. HDL-cholesterol and total cholesterol unknowns were run in duplicates and mean values were used in statistical analysis. Any sample deviating more than 10% was analyzed again to ensure the accuracy of the measurement. Two samples deviating more than 10% could not be run again since there was not enough plasma available for these individuals. The percentage error associated with runs on the control samples for total cholesterol was 3.29% for Fisher standards and 1.03% for pooled plasma samples. The percentage error associated with runs on

the control samples for HDL-cholesterol was 7% for the Fisher standard and 5% for the pooled plasma samples.

## Statistical Analysis

The Statistical Analysis System (SAS) was used for determining associations between dietary, plasma and red blood cell zinc and copper levels. Statistical analyses were performed in three phases. The first phase included the computation of descriptive statistics (mean, standard deviation, maximum and minimum value) for each age and race category and for all girls combined. The second phase included ANOVA and ANCOVA to detect differences in race, socioeconomic status and nutritional status. The third phase included correlation analyses with dietary, plasma and red blood cell zinc and copper levels as well as HDL-cholesterol and total cholesterol, and correlation analyses between total score from the food frequency questionnaire and the average value for the nutrient variables (saturated fat, animal fat, total fat, and cholesterol) obtained from the two 24-hour recalls.

# CHAPTER 4

# RESULTS AND DISCUSSION

The results of this dissertation research are presented in tables 3 through 10. The means, standard deviations, and sample sizes are presented for the cross sectional data as well as the longitudinal data. Exact p-values are presented for all statistical analyses when statistical significance approaches $p < 0.05$.

The results of the cross sectional data on the trace mineral status of the adolescent females are presented first. The cross-sectional data on the relationships of trace mineral status and lipid measures are presented second. The data on the dietary assessment of cholesterol and fat intake with the 24-hour recall method and the food frequency questionnaire are presented third. Finally the data on the longitudinal changes in lipid profiles are presented.

## Zinc Status

Mean dietary zinc intake for the population of all the girls studied was 81% of the Recommended Dietary Allowance for this age group (Table 3). Approximately

Table 3

Dietary Zinc of Adolescent Females

| Group | Dietary Zinc mg/day | Mg Zn/ 1000 kcal | Percentage of RDA |
|---|---|---|---|
| All Girls | 12.1 ± 7.4 n=92 | 5.0 ± 1.9 n=92 | 81 ± 49 n=92 |
| Whites | 13.1 ± 8.1 n=56 | 5.5 ± 2.0 n=56 | 87 ± 54 n=56 |
| Blacks | 10.6 ± 6.0 n=36 | 4.2 ± 1.4 n=36 | 71 ± 40 n=36 |
| 14-year-olds | 12.1 ± 7.0 n=49 | 5.1 ± 1.5 n=49 | 81 ± 44 n=49 |
| 16-year-olds | 12.2 ± 8.3 n=43 | 4.9 ± 2.2 n=43 | 81 ± 55 n=43 |
| Whites, 14 yrs | 12.6 ± 6.6 n=32 | 5.4 ± 1.4 n=32 | 84 ± 44 n=32 |
| Blacks, 14 yrs | 11.1 ± 6.9 n=17 | 4.3 ± 1.3 n=17 | 74 ± 45 n=17 |
| Whites, 16 yrs | 13.8 ± 9.9 n=24 | 5.5 ± 2.5 n=24 | 92 ± 66 n=24 |
| Blacks, 16 yrs | 10.1 ± 5.2 n=19 | 4.1 ± 1.5 n=19 | 68 ± 34 n=19 |

Note. Mean ± standard deviation.

one third of the adolescent females in this study consumed less than 2/3 of the RDA for zinc. These data suggest that the dietary zinc intake of these adolescent females was poor. Black females in this population consumed less zinc per day (11 ± 6.0 mg/day) compared to white females (13 ± 8.1 mg/day); however, this difference was not statistically significant. A significant difference in zinc intake per 1000 kcals between the races ($p$ = 0.001) was observed . White girls consumed 5.48 mg/1000 kcals/day of zinc whereas blacks consumed 4.22 mg/1000 kcals/day of zinc. After covariant adjustment for per capita income mean zinc intake was still significantly different between black and white females (5.47 mg/1000 kcals/day vs. 4.23 mg/1000 kcal/day; $p$ = 0.004). Hypothesis 7, which stated that there would not be differences in zinc and copper status between race or age groups, was rejected at the $p < 0.05$ level.

Sandstead (1982) has suggested that a zinc concentration of <65 ug/100 ml in plasma is indicative of marginal zinc status for females. In this group of adolescent females, 10% were marginally deficient in zinc, i.e. their plasma zinc was ≤65 ug/100 ml (Table 4). Blacks had a higher incidence of marginal plasma zinc status (14%) compared to whites (7%). The

Table 4

Zinc Blood Concentrations of Adolescent Females

| Group | Plasma Zinc ug/100 ml (n) | RBC Zinc ug/g RBC (n) |
|---|---|---|
| All Girls | 84.12 ± 13.86 (91) | 9.47 ± 1.65 (91) |
| Whites | 85.95 ± 15.16 (56) | 9.53 ± 1.34 (56) |
| Blacks | 81.20 ± 11.09 (35) | 9.39 ± 2.07 (34) |
| 14-year-olds | 84.04 ± 12.95 (48) | 9.17 ± 1.53 (48) |
| 16-year-olds | 84.21 ± 14.97 (43) | 9.83 ± 1.72 (42) |
| Whites, 14 yrs | 85.03 ± 14.60 (32) | 9.51 ± 1.46 (32) |
| Blacks, 14 yrs | 82.06 ± 8.88 (16) | 8.48 ± 1.49 (16) |
| Whites, 16 yrs | 87.17 ± 16.09 (24) | 9.56 ± 1.21 (24) |
| Blacks, 16 yrs | 80.47 ± 12.86 (18) | 10.19 ± 2.21 (18) |

Note. Mean ± standard deviation.

incidence of marginal plasma zinc status (10%) was not different between the age groups. Kenny et al. (1984) have suggested that an erythrocyte zinc concentration of less than 8.0 ug/g of RBC is indicative of marginal zinc status. For this group of adolescent females, 22% were marginally deficient in zinc status on the basis of erythrocyte zinc concentrations of less than 8.0 ug/g of RBC. No differences were noted in the incidence of RBC zinc deficiency between the races or age groups. Hypothesis 1, which stated that less than 5% of the adolescent females would be marginally deficient in zinc status, was rejected at the $p < 0.05$ level.

## Copper Status

The dietary copper intake for the adolescent females in this study averaged 1.4 $\pm$ 0.7 mg/day (Table 5). No recommended dietary allowances have been established for the trace element copper; however, a daily intake of 2 to 3 mg has been suggested as being a safe range of intake with a margin of safety for healthy adults (Recommended Dietary Allowances, 1980). In this study the majority (70%) of the adolescent females consumed less than 2/3 of the suggested intake. These findings suggest that the dietary copper intake of these adolescents was relatively poor. Hypothesis 2, which

Table 5

Dietary Copper of Adolescent Females

| Group | Dietary Copper mg/day (n) | Percentage of Suggested Intake (n)[a] |
|---|---|---|
| All Girls | 1.36 ± 0.69 (92) | 54 ± 28 (92) |
| Whites | 1.39 ± 0.65 (56) | 55 ± 26 (56) |
| Blacks | 1.30 ± 0.75 (36) | 52 ± 30 (36) |
| 14-year-olds | 1.34 ± 0.67 (49) | 54 ± 27 (27) |
| 16-year-olds | 1.38 ± 0.72 (43) | 55 ± 29 (29) |
| Whites, 14 yrs | 1.30 ± 0.54 (32) | 52 ± 22 (32) |
| Blacks, 14 yrs | 1.42 ± 0.87 (17) | 57 ± 35 (17) |
| Whites, 16 yrs | 1.53 ± 0.78 (24) | 61 ± 31 (24) |
| Blacks, 16 yrs | 1.19 ± 0.62 (19) | 48 ± 25 (19) |

Note. Mean ± standard deviation.

[a]Percentage based on 2.5 mg/day.

stated that less than 5% of the adolescent females would be marginally deficient in dietary copper, was rejected at the $p < 0.05$ level.

Engel, Price, and Miller (1967) have reported that preadolescent females remain in equilibrium or maintain a slightly positive copper balance on 1.6-2.1 mg of copper per day. Engel et al. (1967) further reported that copper intakes of 1.0 - 1.3 mg/day resulted in negative copper balance for preadolescent females.

Greger et al. (1978) cited that a plasma copper concentration of <70 ug/100 ml was considered marginal copper status. None of the adolescent females in this study were marginally deficient in copper status on the basis of plasma concentrations; however, the majority of the adolescent females in this study consumed a diet rather poor in copper. This discrepancy between the findings for the two measures of copper status may be related to the inaccuracy of assessing dietary copper with two 24-hour recalls or it may be related to the possible unaccounted for contribution of dietary copper from water intake.

Plasma copper concentrations were higher among blacks than whites (114 $\pm$ 21 ug/100 ml vs. 108 $\pm$ 22 ug/100 ml); however, this difference was not statistically significant (Table 6). The Hematological

Table 6

Copper Blood Concentrations of Adolescent Females

| Group | Plasma Copper ug/100 ml (n) | RBC Copper ug/100 ml RBC (n) |
|---|---|---|
| All Girls | 110.3 ± 21.7 (89) | 125.0 ± 16.2 (89) |
| Whites | 108.0 ± 22.2 (55) | 122.6 ± 17.1 (56) |
| Blacks | 113.9 ± 20.6 (34) | 129.0 ± 13.7 (33) |
| 14-year-olds | 106.8 ± 14.8 (48) | 122.0 ± 16.0 (47) |
| 16-year-olds | 114.3 ± 27.4 (41) | 128.4 ± 15.8 (42) |
| Whites, 14 yrs | 103.3 ± 12.1 (32) | 121.5 ± 18.0 (32) |
| Blacks, 14 yrs | 113.9 ± 17.5 (16) | 123.1 ± 11.0 (15) |
| Whites, 16 yrs | 114.6 ± 30.6 (23) | 124.2 ± 16.1 (24) |
| Blacks, 16 yrs | 113.9 ± 23.6 (18) | 133.9 ± 14.0 (18) |

Note. Mean ± standard deviation.

and Nutritional Biochemistry Reference Data from NHANES II indicates similar racial differences in serum copper for adolescent females (Fulwood, Johnson, Bryner, Gunter, & McGrath, 1982). Plasma copper concentrations were higher in the 16-year-old age group (114 ± 27 ug/100 ml) than the 14-year-old age group (107 ± 15 ug/100 ml). This increase in copper concentrations, although not significant, may reflect changes in circulating levels of estrogen (Greger et al., 1978).

Measurement of erythrocyte copper concentrations has been done infrequently in the past due to the necessity of wet ashing (Solomons, 1979). Copper is distributed approximately equally between plasma and erythrocytes and the average concentration of copper in whole blood is 100 ug per 100 ml volume (Li & Vallee, 1980). Actual copper concentrations in erythrocytes of adolescent females have not been reported before; however, Williams, Atkin, Frens, and Bray (1977) have suggested a range of 63 - 107 ug/dl of RBC as normal for healthy adults. Underwood (1978) suggested a value of 115 ± 22 mg/% as normal for copper concentrations in erythrocytes of healthy adults. Dietary deficiency of copper in pigs results in a reduction in copper concentrations of both plasma and erythrocytes but the reduction is greater in plasma than erythrocytes

(Underwood, 1978). Pregnancy in humans results in elevated plasma copper levels while erythrocyte levels remain normal (Underwood, 1978).

## Zinc and Copper Relationships

No significant correlations were observed between the different parameters for assessing zinc status (e.g., plasma, RBC zinc or dietary zinc). Furthermore, no significant correlations were observed between the different parameters of assessing copper status (plasma copper, RBC copper or dietary copper). This lack of significant correlations between measures of zinc status is consistent with findings from other similar studies (Solomons, 1979). Hypothesis 3, which stated that there would be no correlation between dietary intakes and blood levels of zinc or copper, could not be rejected at the $p < 0.05$ level.

A significant inverse relationship ($r = -0.32$, $p = 0.002$) was observed for plasma zinc and RBC copper (Table 7). This relationship has not been reported before perhaps because very few studies have directly measured RBC copper concentrations. The physiological significance of this inverse relationship is unclear but probably reflects the antagonistic effect of zinc on copper absorption and metabolism.

Table 7

RBC Copper Correlations in Adolescent Females

| Variable | RBC Copper (r) | p value |
|---|---|---|
| Plasma Zinc | - 0.32 | 0.002 |
| Plasma Zinc/Copper | - 0.30 | 0.005 |
| Dietary Zinc/Copper | - 0.21 | 0.043 |
| Dietary Zinc/1000 kcals | - 0.19 | 0.070 |

A significant inverse relationship ($r$ = -0.30, $p$ = 0.005) was observed between the plasma ratio of zinc/copper and RBC copper concentrations. Again, this relationship may reflect the antagonistic effect of zinc on copper.

A significant inverse relationship ($r$ = -0.21, $p$ = 0.04) was observed for the dietary zinc/copper ratio and RBC copper. This relationship also supports the antagonistic action of zinc on copper during absorption and metabolism. A high dietary zinc/copper ratio may be detrimental to RBC copper status. A similar relationship to the above between zinc intake/1000 kcals and RBC copper was also observed, i.e., dietary intake of zinc/1000 kcals was inversely related ($r$ = -0.19, $p$ = 0.07) to RBC copper concentrations. Fischer et al. (1979) observed a similar relationship to the above; they found serum copper levels were inversely proportional to dietary zinc levels. Sandstead (1978) has shown that zinc competes with copper for binding to metallothein which aids in the absorption of both copper and zinc. Excessive zinc intake would therefore inhibit absorption of copper. Burke, DeMicco, Taper, and Ritchey (1981) have shown that copper retention (intake minus fecal excretion) was significantly higher ($p$ < 0.05) for elderly individuals consuming a mean of 7.80

mg of zinc/day compared to individuals consuming a mean of 23.26 mg of zinc/day.

## Cross-Sectional Lipid Relationships

Total cholesterol was significantly higher in the 16-year-old females than the 14-year-old females (205 ± 32 mg/100ml vs. 191 ± 34 mg/100ml, $p = 0.04$). HDL-cholesterol was also significantly higher in the 16 year old females than the 14-year-old females (64 ± 11 mg/100 ml vs. 58 ± 12 mg/100 ml, $p = 0.02$). Significant differences in total cholesterol between the age groups were observed in the Lipids Research Clinic Study; however, Liebman et al. (1984) observed no significant differences in total cholesterol between the 12, 14, or 16-year-old adolescent females.

Total cholesterol was not significantly different between the races (197 ± 34 mg/100 ml for whites, 198 ± 34 mg/100 ml for blacks). HDL-cholesterol was significantly different between the races with blacks having a higher HDL-cholesterol level (66 ± 13 mg/100 ml vs. 58 ± 11 mg/100 ml, $p = 0.0009$). Liebman et al. (1984) cited that a higher HDL-cholesterol level in blacks is a consistent finding with other cross-sectional studies of adolescent females. The reason for the higher HDL-cholesterol level in blacks is unknown.

Hypothesis 8, which stated that there would be a significant difference in HDL-cholesterol between the races, could not be rejected at the $p < 0.05$ level.

The total cholesterol for one third of the adolescent females in this study was over the 95th percentile (>201 mg/100 ml) for total cholesterol levels according to the Prevalence Study of the Lipid Research Clinics Program. Hypothesis 4, which stated that less than 5% of the adolescent females would exhibit elevated cholesterol concentrations, was rejected at the $p < 0.05$ level. The high mean value (197 $\pm$ 33 mg/100 ml) for total cholesterol in this study as compared to cholesterol concentrations reported for the Lipid Research Clinics population might be explained by the fact that an Autoanalyzer System was used to measure concentrations in the Lipids Program whereas a manual method was used in this study. Differences in these procedures may account for some of the differences observed in mean values for total cholesterol.

## Trace Mineral and Lipid Relationships

A significant positive relationship ($r = 0.29$, $p = 0.006$) was observed between copper intake from supplements and total cholesterol concentrations. The physiological significance of this relationship is

unknown. Furthermore, this relationship is not consistent with the associations implied by Klevay's (1973) hypothesis.

No significant relationships were observed between zinc or copper parameters and plasma lipid measures in this study. Hypothesis 5, which stated that there would be a relationship between lipid parameters and trace mineral concentrations, was rejected at the $p < 0.05$ level. The dietary zinc/copper ratio in this study was 9:1. Studies by Klevay (1973) reported relationships between dietary zinc/copper and plasma cholesterol when the dietary zinc/copper ratio was 40:1. The lack of associations between zinc/copper ratios and lipid measures in this study may be a reflection of the relatively low dietary zinc/copper. A dietary zinc/copper ratio in excess of 35 is thought to detrimentally affect lipid status (Klevay, 1973).

Food Frequency, 24-Hour Recall and Lipid Relationships

A total score was computed from the food frequency questionnaire for each participant. A high score indicated a high consumption of saturated fat and dietary cholesterol relative to someone receiving a low score on the food frequency questionnaire. A significant positive relationship ($r = 0.26$, $p = 0.012$)

was observed between the total score on the food frequency questionnaire and the total dietary fat intake estimated from the average of two 24-hour recalls. A significant positive relationship ($r$ = 0.32, $p$ = 0.002) was observed between the total score on the food frequency questionnaire and total dietary animal fat intake estimated from the average of two 24-hour recalls. A significant positive relationship ($r$ = 0.21, $p$ = 0.037) was observed between the total score on the food frequency questionnaire and total saturated fat intake. The relationship ($r$ = 0.17, $p$ = 0.11) between the total score on the food frequency questionnaire and dietary cholesterol was not significant.

No significant correlations were observed between dietary parameters estimated from the 24-hour recall and plasma total cholesterol; however, a positive relationship ($r$ = 0.20, $p$ = 0.05) between HDL-cholesterol and total fat intake was observed. An nonsignificant relationship ($r$ = 0.18, $p$ = 0.09) was observed between HDL-cholesterol and total dietary saturated fat intake. HDL-cholesterol for the females was positively related to total cholesterol ($r$ = 0.41, $p$ = 0.0001). The total score on the food frequency questionnaire was somewhat related ($r$ = 0.19, $p$ = 0.07) to HDL-cholesterol concentrations in the females. These

weak relationships between total score on the food frequency questionnaire with dietary fat and HDL-cholesterol suggests a need for further investigation of food frequency questionnaires and lipid measures in various populations, especially in conjunction with more reliable and valid instruments of measuring food intake such as the 3-7 day food record. Instruments such as the food record provide a more reliable and valid indication of one's true eating habits than the 24-hour recall (Bazzarre & Myers, 1982).

## Longitudinal Changes

Body weight and the Quetelet Index for the adolescent females increased significantly during the two year study period (Table 8). Body weight increased 5.63 kg ($p < 0.0001$) and the Quetelet Index increased 0.009 units ($p < 0.0001$). Body weight increased 7.66 kg ($p < 0.0001$) and the Quetelet Index increased 0.012 units ($p < 0.0001$) for those girls who were 12 years old in 1981 (Table 10). Body weight increased 3.32 kg ($p = 0.0004$) and the Quetelet Index increased 0.007 units ($p = 0.052$) for those girls who were 14 years old in 1981 (Table 9).

Total cholesterol and HDL-cholesterol increased significantly for the adolescent females in the study

Table 8

Changes in Body Weight, Quetelet Index, Total Cholesterol, HDL-Cholesterol and the HDL/TC Ratio in Adolescent Females Over a Two-Year Period

| Variable | Mean Changes (1983-1981) (n) | p value |
|---|---|---|
| Body Weight (kg) | 5.63 ± 0.55 (92) | <0.0001 |
| Quetelet Index ($100 \times Ht/Wt^2$) | 0.0095 ± .0019 (92) | <0.0001 |
| Total Cholesterol (mg/100 ml) | 19.5 ± 3.49 (78) | <0.0001 |
| HDL-Cholesterol (mg/100 ml) | 6.1 ± 1.27 (74) | <0.0001 |
| HDL/TC | -0.09 ± 0.34 (74) | 0.78 |

Note. Mean ± standard deviation.

Table 9

Changes in Body Weight, Quetelet Index, Total Cholesterol, HDL-Cholesterol and the HDL/TC Ratio in 14-Year-Old Adolescent Females Over a Two-Year Period

| Variable | Mean Changes (1983-1981) (n) | p value |
|---|---|---|
| Body Weight (kg) | 3.32 ± 0.87 (43) | 0.0004 |
| Quetelet Index ($100 \times Ht/Wt^2$) | 0.0067 ± .0033 (43) | 0.052 |
| Total Cholesterol (mg/100 ml) | 34.6 ± 5.0 (36) | <0.0001 |
| HDL-Cholesterol (mg/100 ml) | 10.6 ± 1.9 (33) | <0.0001 |
| HDL/TC | 0.36 ± 0.13 (33) | 0.01 |

Note. Mean ± standard deviation.

Table 10

Changes in Body Weight, Quetelet Index, Total Cholesterol, HDL-Cholesterol and the HDL/TC Ratio in 12-Year-Old Adolescent Females Over a Two-Year Period

| Variable | Mean Changes (1983-1981) (n) | p value |
|---|---|---|
| Body Weight (kg) | 7.66 ± 0.55 (49) | <0.0001 |
| Quetelet Index ($100 \times Ht/Wt^2$) | .0119 ± .0020 (49) | <0.0001 |
| Total Cholesterol (mg/100 ml) | 6.6 ± 3.9 (42) | 0.10 |
| HDL-Cholesterol (mg/100 ml) | 2.5 ± 1.5 (41) | 0.11 |
| HDL/TC | -0.49 ± 0.59 (41) | 0.45 |

Note. Mean ± standard deviation.

(Table 8). Total cholesterol increased 19.5 mg/100 ml ($p < 0.0001$) and HDL-cholesterol increased 6.1 mg/100ml ($p < 0.0001$). The HDL/TC ratio for the adolescent females decreased 0.09 units although this decrease was not significant.

Total cholesterol and HDL-cholesterol increased significantly for those girls who were 14 years old at the beginning of the study (Table 9). Total cholesterol increased 34.61 mg/100 ml ($p < 0.0001$) and HDL-cholesterol increased 10.64 mg/100 ml ($p < 0.0001$) for those girls who were 14 years old in 1981. The HDL/TC ratio for the 14-year-olds in 1981 increased significantly by 0.3552 units ($p = 0.01$).

Total cholesterol, HDL-cholesterol, and the HDL/TC ratio for those girls who were 12 years old in 1981 did not change significantly during the study (Table 10). This lack of significant change for these girls may be associated with the attainment of menarche. Liebman et al. (1984) cited that cross sectional studies have shown a slight decrease or no increase in total cholesterol levels during puberty in females. Liebman et al. (1984) also cited that the average age of menarche in the U.S. is 12.4 years. Those females in this study who were 12 years old in 1981 may have experienced a slowing of the increase in total cholesterol and HDL-cholesterol due to

hormonal changes during this time. Those girls 14 years old in 1981 were past the age where total cholesterol levels may have decreased.

Changes in total cholesterol were positively correlated ($r$ = 0.43, $p < 0.0001$) with changes in HDL-cholesterol during the two-year period for all females. Changes in body weight were negatively correlated ($r$ = -0.23, $p$ = 0.05) with changes in HDL-cholesterol during the two-year period for all females. Changes in body weight were negatively correlated ($r$ = -0.37, $p$ = 0.05) with changes in HDL-cholesterol for the blacks. No significant correlation was observed between body weight changes and HDL-cholesterol changes during the 2-year period for the whites. The reasons for associations (blacks) or lack of associations (whites) of HDL and body weight between the races cannot be explained but may relate to some genetic difference.

A significant positive relationship ($r$ = 0.34, $p$ = 0.04) was observed between changes in Quetelet Index and changes in total cholesterol for the girls who were 14 years old in 1981. Hypothesis 6, which stated that there would be a correlation between changes in Quetelet Index and changes in total cholesterol, was accepted at the $p < 0.05$ level. Liebman et al. (1984) cited that relationships between plasma lipid concentrations and

body composition measurements have been previously reported in studies of adolescent populations. This relationship between changes in Quetelet Index and total cholesterol suggests that increases in total cholesterol during this time period may be associated with increases in body fat since the Quetelet Index is correlated with percent body fat (Roche, Siervogel, Chumlea, & Webb, 1981). This relationship may not have existed for the younger female group because many of them were experiencing menarche during which time estrogen levels may fluctuate and affect cholesterol levels.

# CHAPTER 5

## SUMMARY

This dissertation research examined the zinc and copper nutritional status of adolescent females and the relationship of zinc and copper status to plasma lipid status in this group. Another research objective was to develop a food frequency questionnaire designed to assess intakes of saturated fat and cholesterol in the diet. In order to better understand the changes in lipid parameters that may occur during adolescence and how these changes may relate to premature development of CHD, a two-year longitudinal assessment of total and HDL-cholesterol was also conducted.

This dissertation research was part of the Southern Regional Nurtition Project: Nutritional Health of Adolescent Females (S-150). Ninety-two healthy adolescent females volunteered to participate in the second phase (1983) of the Southern Regional Nutrition Project. For longitudinal investigations only those females who also participated in the first phase of S-150 during 1981 were included in analyses.

Participants were interviewed at home approximately two weeks before attending the major data collection session at UNC-Greensboro. The two 24-hour recalls were collected approximately two weeks apart, one recall taking place at the home of the volunteer and another at the Nutrition Research Center, UNC-Greensboro. The food frequency questionnaire, which was designed to assess the intake of high cholesterol and high saturated fat foods, was administered during the data collection sessions on Saturday mornings.

Approximately 35 mls of blood were obtained from each of the girls following a minimum 12 hour fast. Plasma and red blood cell aliquots for zinc and copper determinations were frozen in polypropylene tubes for later analysis. HDL-cholesterol and total cholesterol were analyzed on fresh samples of plasma obtained from the original 35 mls of blood. Zinc and copper plasma and red blood cell samples were later thawed and were determined using an atomic absorption spectrophotometer.

All dietary information from the two 24-hour recalls was coded and sent to the Department of Experimental Statistics at Louisiana State University. The Nutrition Analysis System maintained within the Experimental Statistics Department was used to calculate the nutrient intake for the adolescents. Dietary

information was coded in SAS format and sent back to UNC-Greensboro. The SAS Program was used for determining statistical associations between all variables in question.

Dietary zinc intake for the adolescent females in this study was 81% of the Recommended Dietary Allowance. One third of these adolescents consumed less than two thirds of the RDA for zinc. A significant difference ( $p = 0.001$) in zinc intake per 1000 kcals was observed between the races. After covariant adjustment for per capita income, mean intake for zinc was still significantly different ( $p = 0.004$) between black and white females. This consistent finding after covariant adjustment suggests a cultural difference in food intake which in turn affects zinc intakes between the races.

Plasma zinc and red blood cell zinc levels did not differ between the races. Ten percent of the adolescent females were marginally deficient in zinc on the basis of plasma zinc levels $\leq 65$ ug/100 ml. Twenty-two percent of the adolescent females were marginally deficient in zinc on the basis of erythrocyte zinc concentrations of less than 8.0 ug/gm of RBC.

The dietary intake of copper for the adolescent females in this study was $1.4 \pm 0.7$ mg/day which is less than the suggested intake for healthy adults (suggested

intake is 2-3 mg/day). None of the individuals in this study were marginally deficient in copper status with all plasma concentrations greater than 70 ug/100 ml. Mean plasma copper concentration was higher among blacks than whites; however, this difference was not significant. Erythrocyte copper concentrations were within the normal limits for healthy adults, but it could not be determined whether these levels were normal for adolescents since no study has been published reporting erythrocyte copper concentrations in adolescent females.

No significant correlations were observed between the different parameters of assessing zinc status (i.e., plasma, RBC, or dietary zinc) nor were significant correlations observed between the different parameters of assessing copper status (i.e., plasma, RBC, or dietary copper). Red blood cell copper, however, was associated with a number of parameters of zinc nutritional status. Red blood cell copper was inversely correlated with plasma zinc ($r$ = - 0.32, $p$ = 0.002) and the ratio of zinc to copper in plasma ($r$ = - 0.30, $p$ = 0.005). Red blood cell copper was also inversely correlated with the ratio of dietary zinc to copper ($r$ = - 0.21, $p$ = 0.04).

Total cholesterol was significantly higher in the

16-year-old females than the 14-year-old females ( $p$ = 0.04) and HDL-cholesterol was also significantly higher in the 16 year old females ( $p$ = 0.02). Total cholesterol was not significantly different between the races, however, HDL-cholesterol was significantly ( $p$ = 0.0009) higher among the black females than the whites females.

No significant relationships were observed between zinc or copper parameters and lipid measures in this study. A possible explanation for the lack of any association between the zinc/copper ratio and total cholesterol is that the dietary zinc to copper ratio in this study was well below those ratios which have been shown to increase total cholesterol.

A significant positive relationship ( $r$ = 0.26, $p$ = 0.012) was observed between the total score on the food frequency questionnaire and the total dietary fat intake estimated from the average of two 24-hour recalls. A similar relationship was observed between total dietary animal fat and total score on the food frequency questionnaire ( $r$ = 0.32, $p$ = 0.002). Total saturated fat intake was also positively correlated with total score on the food frequency questionnaire ( $r$ = 0.21, $p$ = 0.04). Total dietary cholesterol was not significantly related to total score on the questionnaire.

No significant correlations were observed between dietary parameters estimated from the 24-hour recall and plasma total cholesterol. The relationship between total fat intake estimated from the average of two 24-hour recalls and HDL-cholesterol approached statistical significance ($r$ = 0.20, $p$ = 0.06).

Total cholesterol and HDL-cholesterol increased significantly for the adolescent females during the two-year period. Total cholesterol increased 19 mg/100 ml ($p < 0.0001$) and HDL-cholesterol increased 6.11 mg/100 ml ($p < 0.0001$). The HDL-TC ratio for the adolescents did not change significantly during the two year period. Changes in body weight were negatively correlated ($r$ = -0.23, $p$ = 0.05) with changes in HDL-cholesterol during the two-year period for all females.

A significant positive relationship ($r$ = 0.34, $p$ = 0.04) was observed between changes in the Quetelet Index and changes in total cholesterol for the females who were 14 years old in 1981. This relationship suggests that increases in total cholesterol during this time period may be associated with increases in body fat.

## Recommendations For Future Research

This dissertation research has provided some useful information concerning the nutritional health of

adolescent females. The rather high prevalence of marginal zinc status in adolescent females suggests a need for challenge studies to diagnose the prevalence of zinc deficiency in this high risk population of individuals. The significant relationships between red blood cell copper and zinc parameters suggests the need for further research in the area of trace mineral interactions. The apparent discrepancy in copper status between results from dietary copper and plasma copper suggests further research into what are the true dietary requirements for copper in adolescents and whether two 24-hour recalls can accurately estimate copper intake. Further research with food frequency questionnaires in conjunction with 3-and 7-day food records may provide more knowledge as to their usefulness in the rapid assessment of dietary patterns. Further research investigating the longitudinal changes in lipid parameters along with the longitudinal changes in hormone levels may provide some explanation for the changes in lipid status during adolescence.

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# APPENDIX A

## Consent Form

## Consent for Participation

I have received an explanation of the nutrition study to be conducted at the University of North Carolina at Greensboro as part of the Southern Regional Nutrition Project: Nutritional Health of Adolescent Females (S-150). The project will be directed by Michael Liebman, faculty member in the Department of Food, Nutrition and Food Service Management in the School of Home Economics.

The study objectives are 1) to assess the nutritional health of adolescent females in the Southern region and 2) to relate the nutritional health of adolescent females to socioeconomic factors, food habits, nutrition knowledge, behavioral characteristics, physiological development, and other appropriate factors.

I understand that I will be asked to answer questions about socioeconomic background (such as education, occupation of parents, etc.), food habits, overall health, and lifestyle. I understand that I will be asked to take tests which are designed to assess my personality and attitudes. I am also aware that I will be asked to donate a urine sample and a blood sample after a short period of fasting. The blood sample will be taken by a qualified blood drawer.

The potential risks of this study (such as fainting, bruising, or infection from the blood drawing, and stress during the interviews and tests) have been explained to me. I understand that I will receive $10.00 for being a subject in this study, payable at the end of my participation.

I understand that I am free to withdraw from the study at any time. I understand that all information will be considered private, will be treated confidentially and will not be revealed so as to cause embarrassment. Dr. Liebman or one of the other members of the research staff will be free to answer any questions I may have regarding this study.

Understanding the above, I agree to participate.

_____

Signature, Subject

Understanding the above, I agree to my daughter's participation.

_____
Signature, Parent or Guardian

_____ _____
Date Signature, Interviewer

_____
Social Security Number

# APPENDIX B

## Food Frequency Questionnaire

## FOOD FREQUENCY

How often do you eat or drink the following foods?

Frequency Scale:

1. Eaten two or more times daily.
2. Eaten once daily.
3. Eaten three or more times per week.
4. Eaten at least once a week.
5. Eaten seldom or never.

| FOOD ITEM | EATING FREQUENCY (Circle appropriate answer) | | | | |
|---|---|---|---|---|---|
| Luncheon meats (bologna, salami, boiled ham etc.) | 1 | 2 | 3 | 4 | 5 |
| Beef products (hamburger, steak, sirloin, etc.) | 1 | 2 | 3 | 4 | 5 |
| Cheese (cheddar, swiss, American & other cheeses) | 1 | 2 | 3 | 4 | 5 |
| Whole milk (not skim) | 1 | 2 | 3 | 4 | 5 |
| Lowfat milk 2% (not skim) | 1 | 2 | 3 | 4 | 5 |
| Cottage cheese (not lowfat) | 1 | 2 | 3 | 4 | 5 |
| Cheese spread (i.e. Velvetta) | 1 | 2 | 3 | 4 | 5 |
| Ice cream (milk shakes, sandwiches, fudge bars, etc.) | 1 | 2 | 3 | 4 | 5 |
| Chicken | 1 | 2 | 3 | 4 | 5 |
| Lamb | 1 | 2 | 3 | 4 | 5 |
| Liver and other organ meats | 1 | 2 | 3 | 4 | 5 |
| Sausage | 1 | 2 | 3 | 4 | 5 |
| Ham | 1 | 2 | 3 | 4 | 5 |
| Hotdogs | 1 | 2 | 3 | 4 | 5 |
| Turkey | 1 | 2 | 3 | 4 | 5 |
| Veal | 1 | 2 | 3 | 4 | 5 |
| Eggs | 1 | 2 | 3 | 4 | 5 |
| Bacon | 1 | 2 | 3 | 4 | 5 |
| Peanuts, cashews, almonds & other nuts | 1 | 2 | 3 | 4 | 5 |

TURN THE PAGE AND CONTINUE

FOOD FREQUENCY



| FOOD ITEM | EATING FREQUENCY (Circle appropriate answer) | | | | |
|---|---|---|---|---|---|
| Cornbread | 1 | 2 | 3 | 4 | 5 |
| Muffins (any kind - blueberry, bran etc.) | 1 | 2 | 3 | 4 | 5 |
| Pancakes | | | | | |
| Waffles | 1 | 2 | 3 | 4 | 5 |
| Danish pastries, sweet rolls, coffee cake, doughnuts | 1 | 2 | 3 | 4 | 5 |
| Egg noodles | 1 | 2 | 3 | 4 | 5 |
| Lard | 1 | 2 | 3 | 4 | 5 |
| Butter | 1 | 2 | 3 | 4 | 5 |
| Margarine | 1 | 2 | 3 | 4 | 5 |
| Sour cream | 1 | 2 | 3 | 4 | 5 |
| Sauces and gravies | 1 | 2 | 3 | 4 | 5 |
| Commercially fried foods such as pot. chips & f.fries | 1 | 2 | 3 | 4 | 5 |
| Cream soups (i.e. cream of mushroom etc.) | 1 | 2 | 3 | 4 | 5 |
| Pies, cakes, puddings or cookies | 1 | 2 | 3 | 4 | 5 |
| Commercial popcorn | 1 | 2 | 3 | 4 | 5 |
| Fish (scallops, shrimp, sardines) | 1 | 2 | 3 | 4 | 5 |
| Other types of fish (cod, flounder, mackeral, salmon, tuna, etc.) | 1 | 2 | 3 | 4 | 5 |